DOCUMENT-IDENTIFIER: US 5888790 A
TITLE: Modified Acyl-ACP desaturase

Detailed Description Text (15):
Further, as indicated above, one of skill in the art would predict that within the .DELTA..sup.9 acyl-ACP desaturase family, amino acid contact residues within the substrate binding groove would be substantially similar, if not identical. The amino acid contact residues identified by the X-ray crystallographic work described in Example 1 are residues M114, L115, T117, L118, P179, T181, G188 and F189. That modification of these residues in a .DELTA..sup.9 acyl-ACP desaturase does, in fact, alter the chain-length and double bond positional specifics of the enzyme was confirmed in the experiments described in Example 2. More specifically preliminary experimental work has revealed that a single amino acid substitution at position 118 (Leu to Phe) in Castor .DELTA..sup.9 acyl-ACP desaturase results in an approximately 10-fold increase in its activity with 16:0-ACP. Thus, one amino acid substitution at a contact residue position can generate an acyl-ACP desaturase with novel and useful properties.

Detailed Description Text (46):
Because the iron center is buried in the interior of the .DELTA..sup.9 desaturase, a substrate cleft lined with hydrophobic residues connecting the surface of the enzyme to the active site was expected to be identified. Indeed, a narrow, very deep channel can be found extending from the surface far into the protein. The channel passes the diiron center on the same side as the proposed oxygen binding site. At the bottom of this channel is found the side chain of L115 and the walls consist of residues W139, T192, Y111, M114, Y191, Q195, P266, T99, and T104. The channel then passes the iron cluster and continues towards the surface with residues Y292, M265, F279, and S283 at the narrow entrance of this cleft. The overall shape of the substrate channel which is bent at the position of the iron cluster facilitates binding of the product, oleoyl-ACP with cis configuration at the double bond.

DOCUMENT-IDENTIFIER: US 5705391 A
TITLE: Modified acyl-ACP desaturase

Detailed Description Text (15):
Further, as indicated above, one of skill in the art would predict that within the .DELTA..sup.9 acyl-ACP desaturase family, amino acid contact residues within the substrate binding groove would be substantially similar, if not identical. The amino acid contact residues identified by the X-ray crystallographic work described in Example 1 are residues M114, L115, T117, L118, P179, T181, G188 and F189. That modification of these residues in a .DELTA..sup.9 acyl-ACP desaturase does, in fact, alter the chain-length and double bond positional specificies of the enzyme was confirmed in the experiments described in Example 2. More specifically preliminary experimental work has revealed that a single amino acid substitution at postion 118 (Leu to Phe) in Castor .DELTA..sup.9 acyl-ACP desaturase results in an approximately 10-fold increase in its activity with 16:0-ACP. Thus, one amino acid substitution at a contact residue position can generate an acyl-ACP desaturase with novel and useful properties.

Detailed Description Text (46):
Because the iron center is buried in the interior of the .DELTA..sup.9 desaturase, a substrate cleft lined with hydrophobic residues connecting the surface of the enzyme to the active site was expected to be identified. Indeed, a narrow, very deep channel can be found extending from the surface far into the protein. The channel passes the diiron center on the same side as the proposed oxygen binding site. At the bottom of this channel is found the side chain of L115 and the walls consist of residues W139, T192, Y111, M114, Y191, Q195, P266, T99, and T104. The channel then passes the iron cluster and continues towards the surface with residues Y292, M265, F279, and S283 at the narrow entrance of this cleft. The overall shape of the substrate channel which is bent at the position of the iron cluster facilitates binding of the product, oleoyl-ACP with cis configuration at the double bond.

Detailed Description Text (75):
As described in Example 1, the crystal structure of castor .DELTA..sup.9 -18:0-ACP desaturase was determined, making it possible to interpret the results on chimeras and mutants in light of the arrangement of the active site. The subunit structure contains a very deep and narrow channel which appears to correspond to the binding site for the stearic acid part of the substrate. The form of the channel imposes a bent conformation of the aliphatic chain at the point where the double bond is introduced, between carbon 9 and 10, corresponding to the cis configuration of the oleic acid product, positioning the potential double bond rather close to the catalytic iron center in the subunit. This substrate binding channel thus sets severe restrictions on the length of the aliphatic chain beyond the introduced double bond which can in part explain the differences in specificity for the enzymes in this family. As can be seen, variants of the enzyme which accept substrates with fewer carbon atoms beyond the double bond, have their binding clefts closed by substitutions of amino acid with bulkier side chains. The amino acids involved in determining the specificity in this part of the binding site are 114-115, 117-118, 179, 181 and 188-189.

Detailed Description Text (77):
From the structure of the binding site in this area it is possible to rationalize some of the results on chimeras and mutants. All the chimeras and mutants involve the determinant 179-189 (actually residues 179, 181, 188-189) and it is thus not surprising to find effects on specificity. Both Chimera 1 and 2 have very little residual activity, probably due to some steric clashes upon their formation. Chimera 1 has this determinant of .DELTA..sup.9 -18:0 ACP desaturase in the deep pocket and also the surface determinant specific for of .DELTA..sup.9 -18:0 ACP desaturase, only one determinant, residues 114-115 and 117-118 specific for of .DELTA..sup.6 -16:0 ACP desaturase and thus the little remaining activity of this chimera is that of a .DELTA..sup.9 -18:0 ACP desaturase. Chimera 2 has the whole determinant of A9-18:0 ACP desaturase in the area of the buried pocket and the known determinant of .DELTA..sup.6 -16:0 ACP desaturase at the surface end; this chimera also has .DELTA..sup.9 -18:0 ACP activity. Chimera 3 and 4 have retained their activity, one of the determinants in the deep pocket is that for a .DELTA..sup.9 -18:0 ACP, residue A181 is substituted for the larger threonine sidechain but at the same time A188 is substituted for glycine and Y189 for phenylalanine, actually making more space available in the deep cavity and thus allowing even for .DELTA..sup.6 -18:0 ACP activity. Chimera 5, differs from chimera 4 only in that it has retained the .DELTA..sup.6 -16:0 ACP

desaturase sequence for residues 203-207. These residues are at the upper part of the substrate channel but do not make direct contact to the substrates and it is difficult to understand the effect on the substrate specificity. These residues are fairly conserved between the known desaturases in this family, only .DELTA..sup.6 -16:0 ACP desaturase has a different sequence for residue 205 to 207, and this region probably does not constitute part of the natural determinant for substrate specificity. In the case of mutant A181T/A200F the decrease in the .DELTA..sup.6 -16:0 ACP activity compared to the wild type enzyme is consistent with the structural changes in the substrate channel due to a decrease in size of this cavity by changing A181 to threonine. The effect of A200F is not possible to rationalize, this residue is on the surface of the subunit pointing away from the substrate-channel. In all sequenced desaturases in this family except .DELTA..sup.6 -16:0 ACP this residue is a phenylalanine. From the foregoing discussion it is clear that the activity of A181T/A200F/S205N/L206T/G207A is impossible to explain in structural terms, we can not rationalize the effects of changes at residues 200 and 205-207.

CLAIMS:

2. The nucleic acid sequence of claim 1 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid contact residues selected from the group consisting of residues 114, 115, 117, 118, 179, 181, 188 and 189.

4. The DNA expression construct of claim 3 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid contact residues selected from the group consisting of residues 114, 115, 117, 118, 179, 181, 188 and 189.

6. The cell of claim 5 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid contact residues selected from the group consisting of residues 114, 115, 117, 118, 179, 181, 188 and 189.

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Friday, October 20, 2006

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L5	M114 and T117 and 118 and P179 and T181 and G188	5
☐	L4	L1 and (Met114 or Thr117 or Leu118 or Pro179 or Thr181 or Gly188)	0
☐	L3	L1 and (M114 or T117 or 118 or P179 or T181 or G188)	36
☐	L2	L1 and (114 or 117 or 179 or 181 or 188)	101
☐	L1	delta-9 desaturase and (mutation? or mutant? or variant?)	125

END OF SEARCH HISTORY

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Friday, October 20, 2006

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☐	L5	M114 and T117 and 118 and P179 and T181 and G188	5
☐	L4	L1 and (Met114 or Thr117 or Leu118 or Pro179 or Thr181 or Gly188)	0
☐	L3	L1 and (M114 or T117 or 118 or P179 or T181 or G188)	36
☐	L2	L1 and (114 or 117 or 179 or 181 or 188)	101
☐	L1	delta-9 desaturase and (mutation? or mutant? or variant?)	125

END OF SEARCH HISTORY

```
=> s M114 and T117 and 118 and P179 and T181 and G188 and desaturase
L1            0 M114 AND T117 AND 118 AND P179 AND T181 AND G188 AND DESATURASE

=> s M114 and T117 and 118 and P179 and T181 and G188
L2            0 M114 AND T117 AND 118 AND P179 AND T181 AND G188

=> s Met114 and Thr117 and leu118 and Pro179 and Thr181 and Gly188
L3            0 MET114 AND THR117 AND LEU118 AND PRO179 AND THR181 AND GLY188

=> s   desaturase and (Met114 or Thr117 or leu118 or Pro179 or Thr181 or Gly188)
L4            0 DESATURASE AND (MET114 OR THR117 OR LEU118 OR PRO179 OR THR181
                OR GLY188)

=> s    (Met114 or Thr117 or leu118 or Pro179 or Thr181 or Gly188)
L5          253 (MET114 OR THR117 OR LEU118 OR PRO179 OR THR181 OR GLY188)

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6            81 DUP REM L5 (172 DUPLICATES REMOVED)

=> s l6 and delta-9
L7            0 L6 AND DELTA-9

=> s delta-9 desaturase and (mutation? or mutant? or variant?)
L8          148 DELTA-9 DESATURASE AND (MUTATION? OR MUTANT? OR VARIANT?)

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9            67 DUP REM L8 (81 DUPLICATES REMOVED)

=> s l9 and castor
L10           3 L9 AND CASTOR

=> d l10 1-3 ibib ab

L10  ANSWER 1 OF 3  HCAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:         2001:455691  HCAPLUS
DOCUMENT NUMBER:          135:177075
TITLE:                    Engineering .DELTA.9-16:0-acyl carrier protein (ACP)
                          desaturase specificity based on combinatorial
                          saturation mutagenesis and logical redesign of the
                          castor .DELTA.9-18:0-ACP desaturase
AUTHOR(S):                Whittle, Edward; Shanklin, John
CORPORATE SOURCE:         Biology Department, Brookhaven National Laboratory,
                          Upton, NY, 11973, USA
SOURCE:                   Journal of Biological Chemistry (2001), 276(24),
                          21500-21505
                          CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER:                American Society for Biochemistry and Molecular
                          Biology
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
AB    Six amino acid locations in the sol. castor .DELTA.9-18:0-acyl
      carrier protein (ACP) desaturase were identified that can affect substrate
      specificity.  Combinatorial satn. mutagenesis of these six amino acids, in
      conjunction with selection, using an unsatd. fatty acid auxotroph system,
      led to the isolation of variants with up to 15-fold increased
      specific activity toward 16-carbon substrates.  The most improved
      mutant, com2, contained two substitutions (T117R/G188L) common to
      five of the 19 complementing variants subjected to further anal.
      These changes, when engineered into otherwise wild-type 18:0-ACP
      desaturase to make mutant 5.2, produced a 35-fold increase in
      specific activity with respect to 16-carbon substrates.  Kinetic anal.
      revealed changes in both kcat and Km that result in an 82-fold improvement
      in specificity factor for 16-carbon substrate compared with wild-type
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enzyme. Improved substrate orientation apparently compensated for loss of binding energy that results from the loss of desolvation energy for 16-carbon substrates. Mutant 5.2 had specific activity for 16-carbon substrates 2 orders of magnitude higher than those of known natural 16-carbon specific desaturases. These data support the hypothesis that it should be possible to reengineer archetypal enzymes to achieve substrate specificities characteristic of recently evolved enzymes while retaining the desired stability and/or turnover characteristics of a parental paralog.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:31112 HCAPLUS
DOCUMENT NUMBER: 128:99301
TITLE: Engineered acyl-ACP desaturases with modified chain length and double bond specificity
INVENTOR(S): Cahoon, Edgar B.; Shanklin, John; Lindgvist, Ylva; Schneider, Gunter
PATENT ASSIGNEE(S): Associated Universities, Inc., USA
SOURCE: U.S., 14 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5705391	A	19980106	US 1996-689823	19960814
US 5888790	A	19990330	US 1997-853979	19970509
CA 2263281	AA	19980219	CA 1997-2263281	19970801
WO 9806735	A1	19980219	WO 1997-US13690	19970801
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9738266	A1	19980306	AU 1997-38266	19970801
AU 718200	B2	20000406		
EP 934332	A1	19990811	EP 1997-935294	19970801
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI				
BR 9711078	A	20000111	BR 1997-11078	19970801
JP 2000516092	T2	20001205	JP 1998-509813	19970801
EE 4020	B1	20030415	EE 1999-63	19970801
RU 2228356	C2	20040510	RU 1999-104591	19970801
NO 9900669	A	19990412	NO 1999-669	19990212
US 6100091	A	20000808	US 1999-276295	19990325
PRIORITY APPLN. INFO.:			US 1996-689823 A2	19960814
			US 1997-853979 A1	19970509
			WO 1997-US13690 W	19970801

AB Disclosed is a methods for modifying the chain length and double bond positional specificities of a sol. plant fatty acid desaturase. More specifically, the method involves modifying amino acid contact residues in the substrate binding channel of the sol. fatty acid desaturase which contact the fatty acid. Amino acid contact residues which lie within the substrate binding channel are identified by alignment with the primary amino acid sequence of the Ricinus communis .DELTA.9 desaturase for max. sequence conservation. A 3-dimensional model for the acyl-[acyl carrier protein] desaturase is then constructed based on the sequence conservation with the R. communis .DELTA. 9 desaturase. A mutant acyl-ACP desaturase

having modified chain length and double bond positional specificity is then generated by replacing one or more of the amino acid contact residues with another amino acid residue. Residues at conserved positions 114, 115, 117, 118, 179, 181, 188, and 189 are most relevant in detg. specificity of the desaturase.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 3 OF 3 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-22320 BIOTECHDS
TITLE: Recombinant polypeptide for transgenic plant for producing seed oil, comprises delta-9 desaturase enzyme from prokaryote in operable linkage with endoplasmic reticulum retention and retrieval signal sequence;
Agrobacterium tumefaciens-mediated gene transfer and expression for transgenic plant construction and propagation
AUTHOR: SHAH S; WESELAKE R
PATENT ASSIGNEE: ALBERTA RES COUNCIL INC
PATENT INFO: WO 2005059140 30 Jun 2005
APPLICATION INFO: WO 2004-CA2156 17 Dec 2004
PRIORITY INFO: CA 2003-2450000 18 Dec 2003; CA 2003-2450000 18 Dec 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-521941 [53]
AB DERWENT ABSTRACT:

NOVELTY - A recombinant polypeptide comprises a delta-9 desaturase enzyme from a prokaryote in operable linkage with an endoplasmic reticulum retention and retrieval signal sequence.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a nucleic acid molecule encoding the recombinant polypeptide; (2) a vector, comprising the nucleic acid molecule in operable linkage with a promoter; (3) a host cell transformed with the above vector; (4) a transgenic plant cell, comprising a transgenic element containing the nucleic acid molecule in operable linkage with the promoter that effects expression of the recombinant polypeptide in the transgenic plant cell; (5) a transgenic plant, comprising the transgenic element containing the nucleic acid molecule in operable linkage with the promoter that effects expression of the recombinant polypeptide in the transgenic plant; (6) use of the transgenic plant for producing seed oil having a reduced saturated fatty acid content as compared to a wild-type plant of the same species; and (7) a method of making the transgenic plant, comprising transforming a plant cell with the nucleic acid molecule or the vector; and regenerating a plant from the transformed plant cell.

BIOTECHNOLOGY - Preferred Component: The delta-9 desaturase enzyme comprises a polypeptide having 278 amino acids given in the specification; a variant or homologue of the above polypeptide having at least 50%, 60%, 70%, 80%, 85%, 90% or 95% identity and having delta-9 desaturase activity; and a fragment of the above polypeptide having greater than or equal to50 contiguous amino acids and having delta-9 desaturase activity. It has 278 amino acids sequence set as given in the specification. The transgenic plant produces oil having a reduced saturated fatty acid content as compared to a wild-type plant of the same species, where the saturated fatty acid content of the seed oil is reduced at 10, 15, 20, 30, 40, 50% or more as compared to the wild-type plant. The saturated fatty acid content is less than 7 mole%. The seed oil has a saturated fatty acid content of 4-4.5%. The endoplasmic reticulum membrane retention and retrieval signal has an amino acid sequence of KDEL (Lys-Asp-Glu-Leu), KKXX (Lys-Lys-Xaa-Xaa), HDEF (His-Asp-Glu-Phe), KEEL (Lys-Glu-Glu-Leu), or KDQL (Lys-Asp-Gln-Leu). It preferably has an amino acid sequence of KKSS (Lys-Lys-Ser-Ser). X = any amino acid;and Xaa = not defined.

USE - For a transgenic plant (claimed).

ADVANTAGE - The inventive polypeptide reduces the saturated fatty acid content in the seed oil produced by the transgenic plant.

EXAMPLE - Canola cultivar 'Wester' was transformed with pC7 and pC8 gene constructs using protocol. In brief, fully unfolded cotyledons from 5 days old seedlings were cut off including petiole with a sharp knife as close to an apical meristem as possible without including it. The cut end of the petiole was dipped briefly into a 1-ml liquid culture of Agrobacterium tumefaciens harboring the des9 gene construct. The petioles were then embedded into Murashige Minimal Organics (MMO)-benzyle adenine (BA) co-cultivation medium in petri plates so that explants stand up vertical. The plates were sealed with surgical tape and kept in growth room at 25degreesC with 16 hours light/8 hours dark, 70-80 mE for 2-3 days. Callus was induced by transferring the explants into MMO-BA medium containing 300 mg/L Timentin.(42 pages)

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=> d his

     (FILE 'HOME' ENTERED AT 14:30:30 ON 20 OCT 2006)

     FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT
     14:31:01 ON 20 OCT 2006
L1             0 S M114 AND T117 AND 118 AND P179 AND T181 AND G188 AND DESATURA
L2             0 S M114 AND T117 AND 118 AND P179 AND T181 AND G188
L3             0 S MET114 AND THR117 AND LEU118 AND PRO179 AND THR181 AND GLY188
L4             0 S   DESATURASE AND (MET114 OR THR117 OR LEU118 OR PRO179 OR THR
L5           253 S    (MET114 OR THR117 OR LEU118 OR PRO179 OR THR181 OR GLY188)
L6            81 DUP REM L5 (172 DUPLICATES REMOVED)
L7             0 S L6 AND DELTA-9
L8           148 S DELTA-9 DESATURASE AND (MUTATION? OR MUTANT? OR VARIANT?)
L9            67 DUP REM L8 (81 DUPLICATES REMOVED)
L10            3 S L9 AND CASTOR

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    26.10      26.31

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                    -1.50      -1.50

STN INTERNATIONAL LOGOFF AT 14:37:57 ON 20 OCT 2006
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Score Home Page Retrieve Application List SCORE System Overview SCORE FAQ Comments / Suggestions

This page gives you Search Results detail for the Application 10822370 and Search Result us-10-822-370-1.rpr.
start

GenCore version 5.1.9

OM protein - protein search, using sw model

Run on: September 25, 2006, 16:42:26 ; Search time 43 Seconds
(without alignments)
812.248 Million cell updates/sec

Title: US-10-822-370-1
Perfect score: 1916
Sequence: 1 ASTLKSGSKEVENLKKPFMP.....RAKEAPTMPFSWIFDRQVKL 363

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 283416 seqs, 96216763 residues

Total number of hits satisfying chosen parameters: 283416

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : PIR_80:*
1: pir1:*
2: pir2:*
3: pir3:*
4: pir4:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	1916	100.0	396	1	OHCSAD	acyl-[acyl-carrier
2	1760	91.9	396	2	B39170	acyl-[acyl-carrier
3	1752	91.4	396	1	A39173	acyl-[acyl-carrier
4	1738	90.7	411	2	T07806	acyl-[acyl-carrier
5	1733	90.4	401	2	E84869	stearoyl-ACP desat
6	1727	90.1	396	2	T14264	acyl-[acyl-carrier
7	1724.5	90.0	399	1	OHSPAD	acyl-[acyl-carrier
8	1701	88.8	396	2	T14268	acyl-[acyl-carrier
9	1698	88.6	398	2	S23351	acyl-[acyl-carrier
10	1680	87.7	399	2	S24995	acyl-[acyl-carrier